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Molecular characterization of the melanocyte lineage-specific antigen gp100.
Adema GJ; de Boer AJ; Vogel AM; Loenen WA; Figdor CG
Division of Immunology, The Netherlands Cancer Institute, Antoni van Leeuwenhoek Huis, Amsterdam.
Journal of biological chemistry (UNITED STATES) Aug 5 1994, 269 (31) p20126-33, ISSN 0021-9258 Journal Code: HIV
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The glycoproteins recognized by monoclonal antibody (mAb) NKI-beteb are among the best diagnostic markers for human melanoma because their expression is restricted to melanocytic cells. Recently, we isolated a cDNA clone, termed **gp100** -c1, which confers immunoreactivity not only to mAb NKI-beteb, but also to two other mAbs used to diagnose malignant melanoma, HMB-50 and HMB-45. In this report, we demonstrate that **gp100** -c1 cDNA encodes glycoproteins of 100 kDa (**gp100**) and 10 kDa (gp10) which are recognized by these mAbs in human melanoma cells. The translation product deduced from the open reading frame present in **gp100** -c1 cDNA is highly homologous to another melanocyte-specific protein, Pmel17. Nucleotide sequence analysis of genomic DNA indicates that the transcripts corresponding to **gp100** and Pmel17 cDNAs originate from a single gene via alternative splicing. In all normal and malignant melanocytic cells analyzed, **gp100** and Pmel17 RNAs are simultaneously expr**8/3,AB/2** **(Item 1 from file: 15**
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Cloning of the gene coding for a shared human melanoma **antigen recognized by autologous T cells infiltrating into tumor.**
Kawakami Y; Eliyahu S; Delgado CH; Robbins PF; Rivoltini L; Topalian SL; Miki T; Rosenberg SA
Surgery Branch, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892.
Proceedings of the National Academy of Sciences of the United States of America (UNITED STATES) Apr 26 1994, 91 (9) p3515-9, ISSN 0027-8424 Journal Code: PV3
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By **cDNA** expression cloning we have isolated a gene encoding a shared human **melanoma** antigen recognized by HLA-A2 restricted autologous and allogenic tumor-infiltrating lymphocytes (TILs) from patients with metastatic **melanoma** . By using both transient and stable expression

systems, transfection of this gene into non-antigen-expressing HLA-A2+ cell lines resulted in recognition by the antigen-specific TILs. The sequence of this **cDNA** revealed a previously undescribed putative transmembrane protein whose expression was restricted to **melanoma** and **melanocyte** cell lines and human retina but no other fresh or cultured normal tissues tested or other tumor histologies. Thus, we have identified a gene encoding a **melanocyte** lineage-specific protein (**MART - 1 ; melanoma** antigen recognized by T cells 1) that is a widely shared **melanoma** antigen recognized by the T lymphocytes of patients with established malignancy. Identification of this gene opens possibilities for the development of immunotherapies for patients with **melanoma** .

T-cell recognition of melanoma **antigens and molecular analysis of the T-cell receptor in patients with different clinical conditions (Meeting abstract).**

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Proc Annu Meet Am Assoc Cancer Res; 36:676-7 1995 ISSN 0197-016X
Languages: ENGLISH
Document Type: MEETING ABSTRACTS

Melanoma lesions are often infiltrated by T lymphocytes to a variable extent and primary tumors are usually more heavily infiltrated than metastatic counterparts. The biologic and therapeutic significance of the infiltrate, however, is still unclear. The purpose of this study was (a) to generate cytotoxic T lymphocyte (CTL) clones recognizing HLA-A2-restricted **melanoma** antigens; (b) to determine the TCR repertoire of CTL recognizing molecularly defined antigens; (c) to see whether T-cell receptors (TCR) associated with recognition of a given antigen are preferentially expressed in the infiltrate of primary and metastatic lesions of HLA-A2 patients and in lesions of vaccinated patients. T-cell clones were obtained by limiting dilution, either from peripheral blood lymphocytes (PBL) or tumor-infiltrating lymphocytes (TIL) of **melanoma** patients, after culture in the presence of autologous tumor and low amount of IL-2. The **melanoma** antigen recognized by CTL clones was identified by using COS-7 cells transfected both with the HLA-A2.1 gene and with genes of Melan-A/**MART -1** , tyrosinase or MAGE-1, to stimulate TNF release by the lymphocyte effectors. TCR repertoire was evaluated by **cDNA** PCR using C-alpha or C-beta primers along with specific primers for each V-alpha and V-beta subfamily. We have generated HLA-A2-restricted CTL clones from **melanoma** patients and have observed an enrichment at the neoplastic site of a CTL clone that recognized a **melanocyte** differentiation antigen shared by HLA-A2+ **melanomas** and **melanocytes** . This suggested that an expansion or accumulation of at least a HLA-A2-restricted clone occurred at the site of tumor growth. A similar finding was reported in a **melanoma** patient whose regressing tumor contained predominantly TCRBV16+ TIL with lytic activity restricted by HLA-B14. Then we analyzed the extent of TCR diversity on a panel of autologous HLA-A2-restricted CTL clones derived from 5 different patients with metastatic **melanomas** . Most clones recognized only Melan-A/ **MART - 1** as evaluated by their ability to release TNF in response to HLA-A2.1 positive COS-7 cells expressing the Melan-A/**MART -1** but not the other antigens. TCRAV2S1 was the predominant alpha chain V region, being transcribed in 6 out of a total of 9 independent Melan-A/**MART -1** specific clones obtained from the 5 different patients. TCRBV region usage was also restricted with either TCRBV14 or TCRBV7 expressed by all but one clone. In addition, a conserved TCRAV2S1/TCRBV14 combination was expressed in 4 CTL clones from 3 patients. None of these V region genes was found in a group of 4 HLA-A2 restricted CTL clones recognizing different antigens (eg, tyrosinase). TCRA1 or TCRBJ usage was heterogeneous, although conserved structural features were observed within the junctional regions. To further evaluate the importance of the HLA-A2 allele in the use of a restricted TCR repertoire and the possible role of the clinical stage, we have examined by semi-quantitative PCR the TCRBV repertoire of TIL from 6 HLA-A2-matched, Melan-A/**MART -1** + primary **melanomas** in comparison with their autologous PBL. T cells using TCRBV14 were over-represented in the neoplastic site of all patients. Analysis of 6 additional primary

melanomas from non-HLA-A2 patients and of normal skin (both HLA-A2 positive or negative), failed to reveal a predominance of TCRBV14 transcripts in such tissues. These results indicate that a selective accumulation of T-cells recognizing at least Melan-A/ **MART -1** by a restricted repertoire of TCR occurs in primary and metastatic **melanoma** lesions. CTL clones deriving from this infiltrate can destroy in vitro both cultured and fresh HLA-A2-compatible **melanoma** cells. This analysis was then extended to **melanoma** lesions of patients vaccinated with irradiated, dinitrophenyl-modified autologous tumor cells. Although the lesions of few patients have been examined so far, three patients' metastases showed an accumulation of TCRBV14. (5 Refs)

08236877 95114402

Generation of tumor-specific CTLs from melanoma patients by using peripheral blood stimulated with allogeneic melanoma tumor cell lines. Fine specificity and MART-1 melanoma antigen recognition.

Stevens EJ; Jacknin L; Robbins PF; Kawakami Y; el Gamil M; **Rosenberg SA**; Yannelli JR

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PBLs were isolated from 13 patients with metastatic melanoma. Mixed lymphocyte tumor cell cultures (ML TCs) were established (15 times) by using irradiated HLA-matched (one class I locus) allogeneic melanoma tumor cell lines (13 times) or autologous melanoma tumor cell lines (two times) in medium containing 120 IU/ml IL-2 and 100 IU/ml IL-4. PBLs grew to levels that could be assessed for functional reactivity 9 of 15 times. In seven of nine cases, CD3+CD8+ CTLs grew from MLTCs that were tumor specific; five were restricted by HLA-A2 and two were restricted by HLA-A24. Four of the tumor-specific CTL lines lysed autologous fresh tumor cells. Tumor-specific CTLs from two of three patients had cytolytic activity identical with tumor-infiltrating lymphocytes (TIL) derived from tumor biopsies removed earlier and grown in high concentrations (6000 IU/ml) of IL-2. Three of the HLA-A2-restricted tumor-specific CTLs were shown to recognize 293 cells transfected with HLA-A2.1 cDNA and the **gene** encoding the melanoma Ag, **MART - 1** . In addition, these CTLs recognized the T2 cell line pulsed exogenously with the peptide MART-1(27-35), which is the nine-amino acid immunodominant epitope of the MART-1 Ag recognized on melanoma tumor cells by nearly all HLA-A2-restricted TIL. Thus, we have demonstrated the ability to generate tumor-specific CTLs from PBLs that are similar in their reactivity to TIL. This technique obviates the need for autologous tumor tissue and suggests that PBLs contain sufficient CTL precursors for use in generating antitumor CTLs for cellular immunotherapy trials.